Epigenetic Mechanisms in Autism Spectrum Disorders

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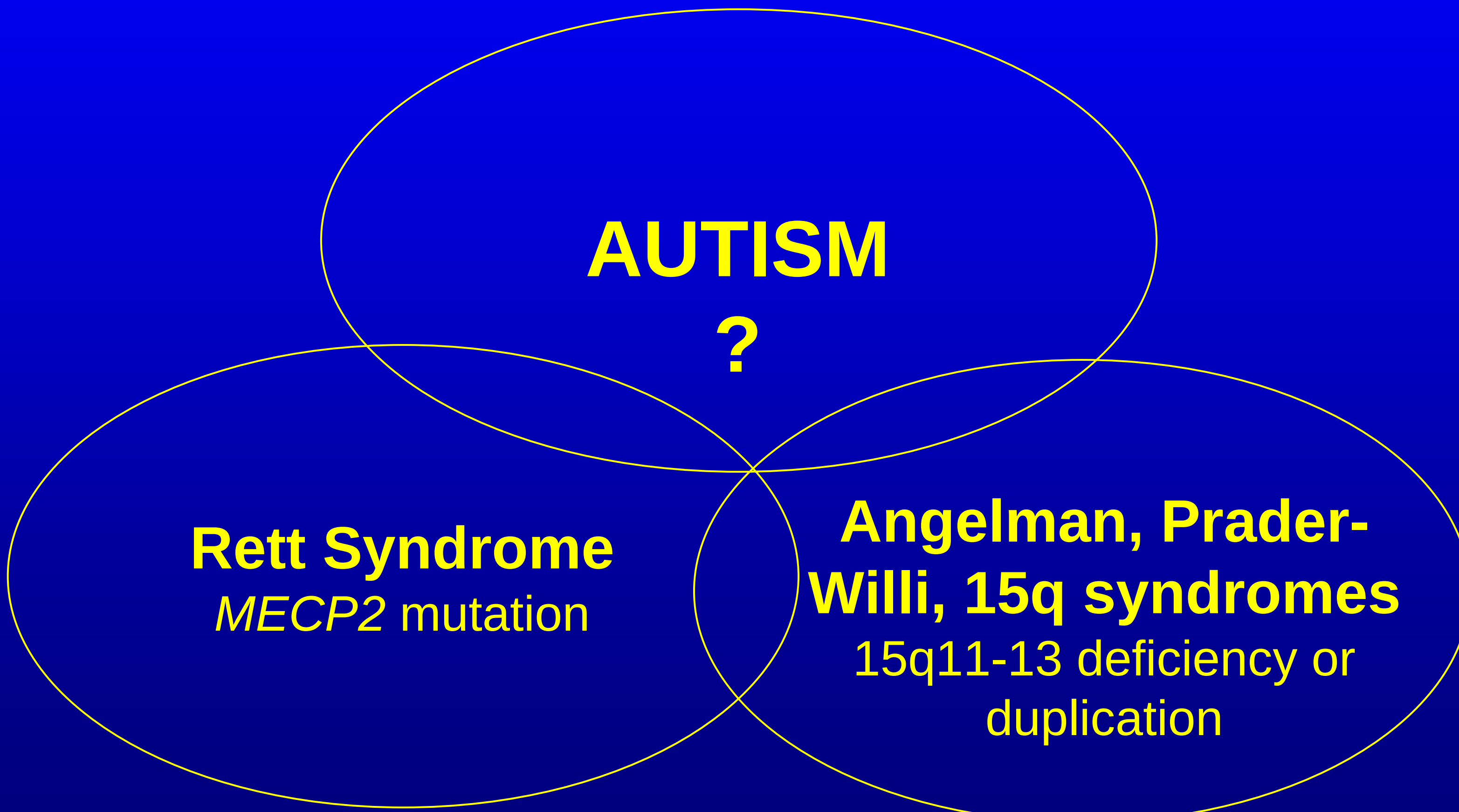
Autism and Epigenetics
What's the connection?



Autism

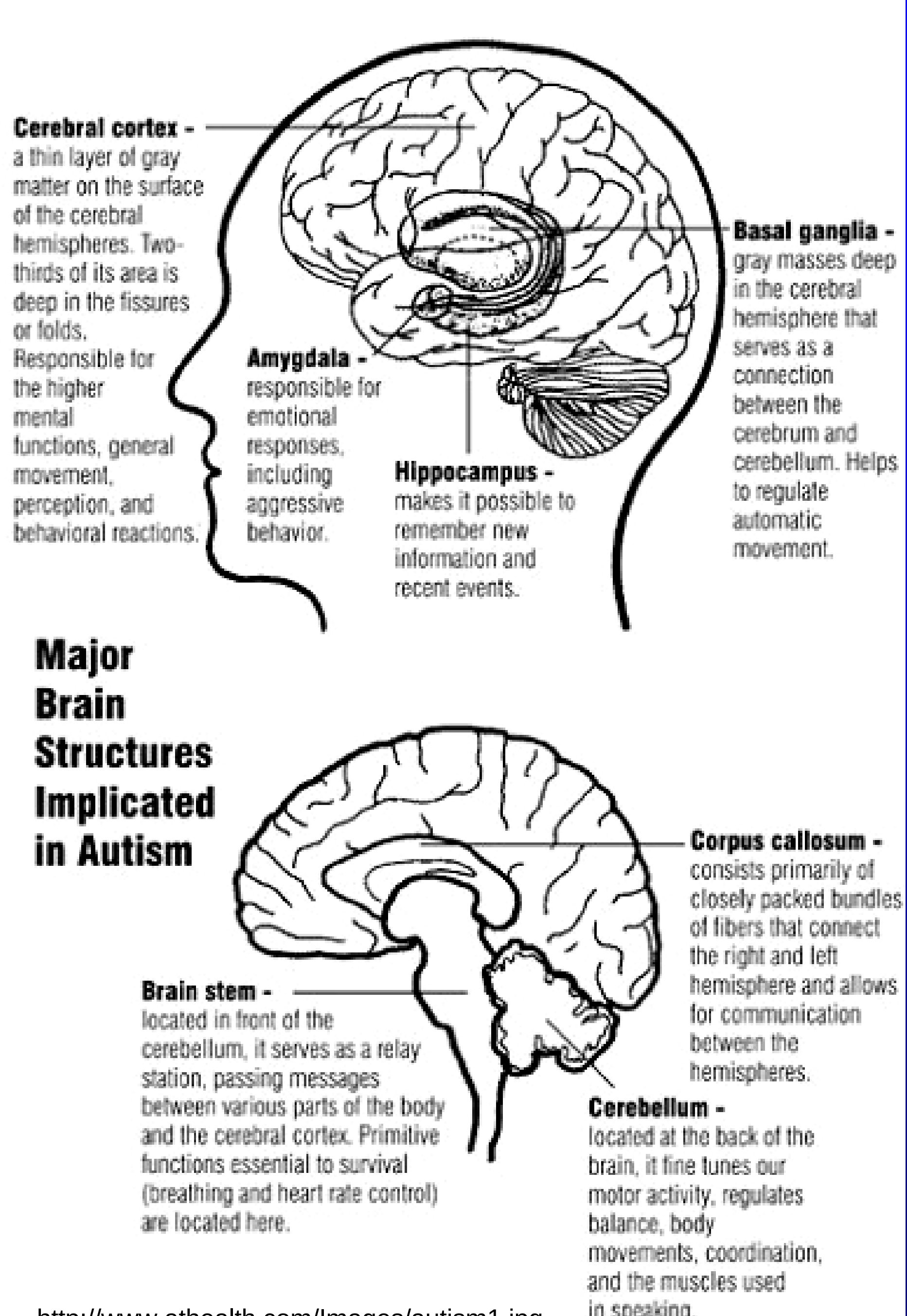
- Complex developmental disorder that usually appears in first three years of life
- Not a single disorder but a spectrum of neurodevelopmental disorders characterized by:
  - Impairments in social interactions and communication
  - Impairments in language
  - Restrictive and repetitive interests and behaviors

Autism

- Regressive autism: apparently normal infancy followed loss of recpirocal social interactions, loss of language, gain of sterotypical behaviors around 18 mo to 4 years of age
- Early onset autism: no apparent loss of language or social interactions

- Male bias for autism 4:1; Asperger’s 10:1

Autism most likely results from alterations in brain development and maturation due to a combination of genetic and environmental factors



http://www.athealth.com/Images/autism1.jpg

Genetics of Autism

- Strong genetic component to risk for autism:
  - Family studies: 50x greater risk for sibs of children with autism compared to the general population.
  - Identical twin studies

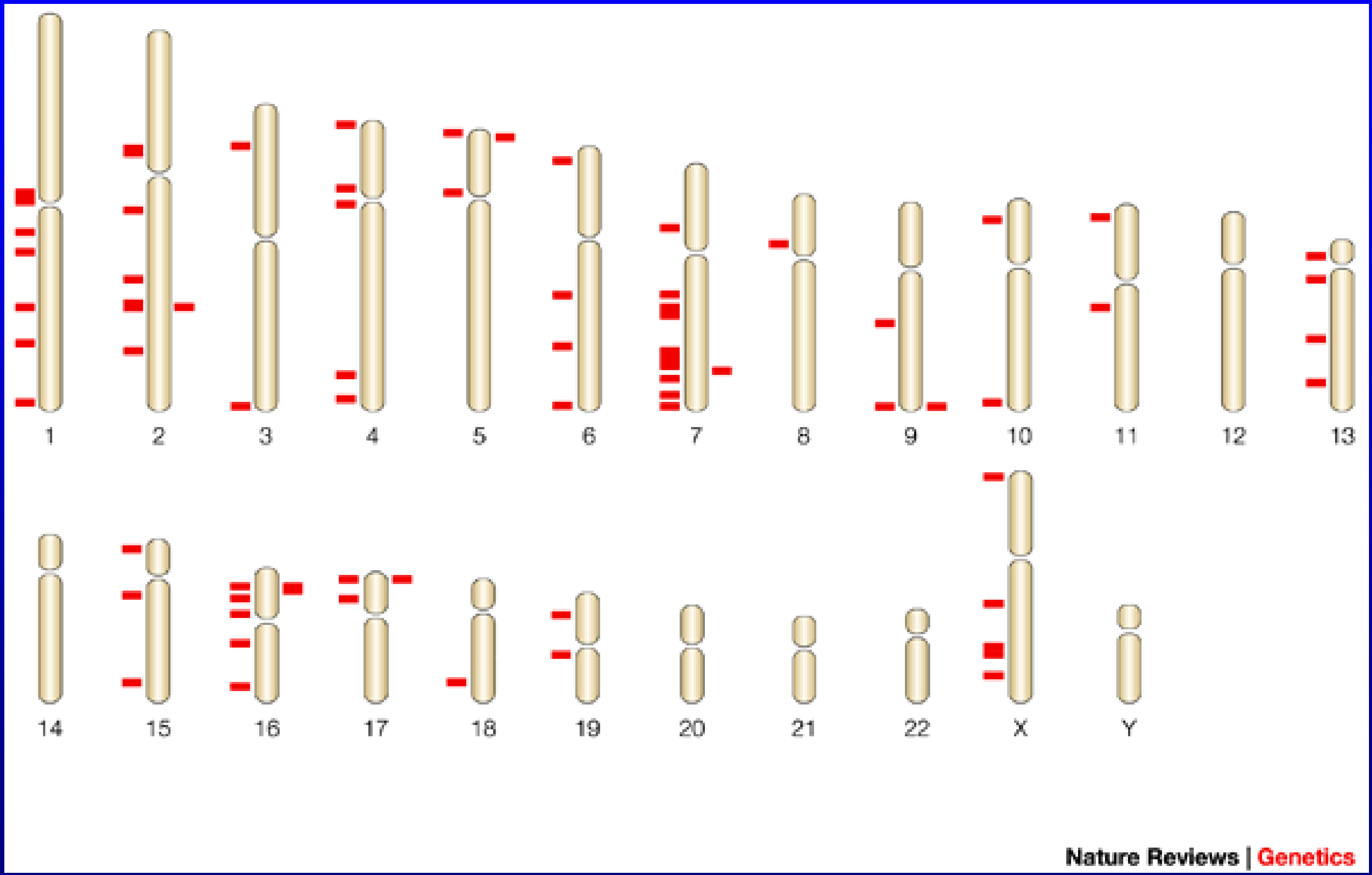
    MZ concordance = 60-90%

    DZ concordance = 0-10 %
  - H^2 **>** 90%

But genetic basis is likely complex;

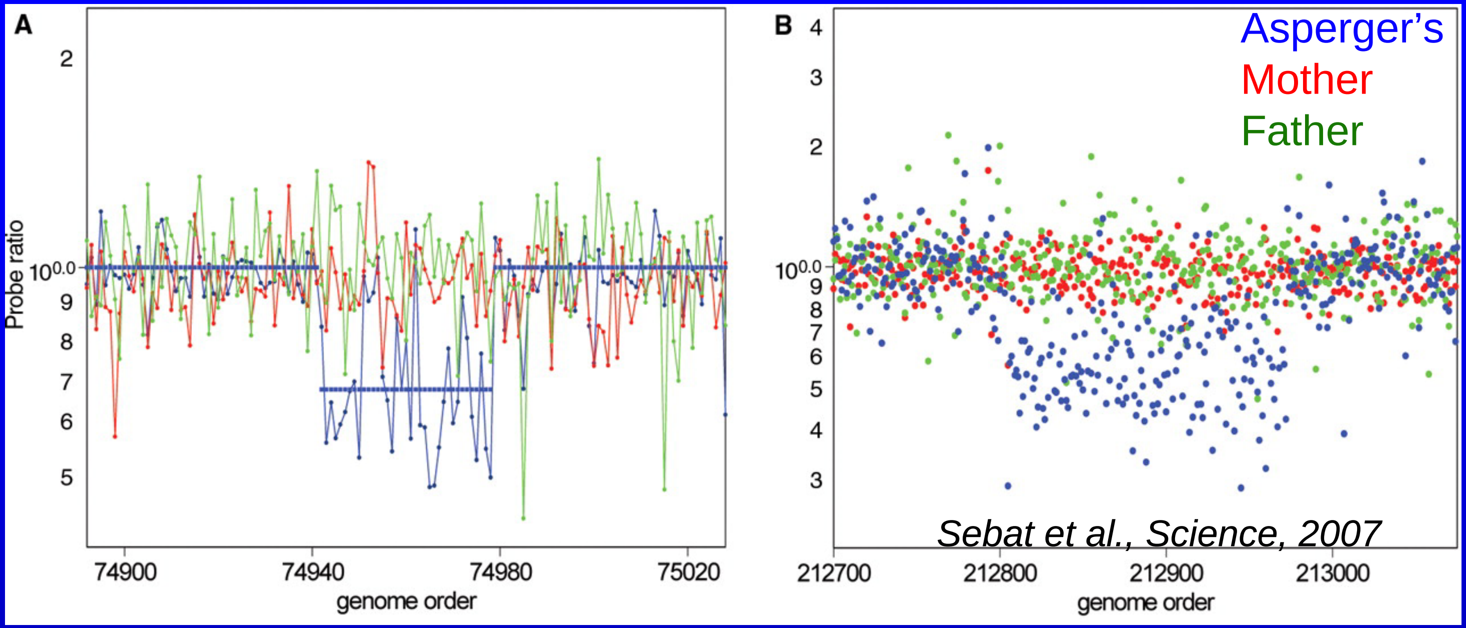
multiple approaches are needed

Loci identified by genome scans that might increase risk of autism



Folstein and Rosen-Sheidley, 2001, Nature Reviews Genetics

New mutations and copy number variations in autism



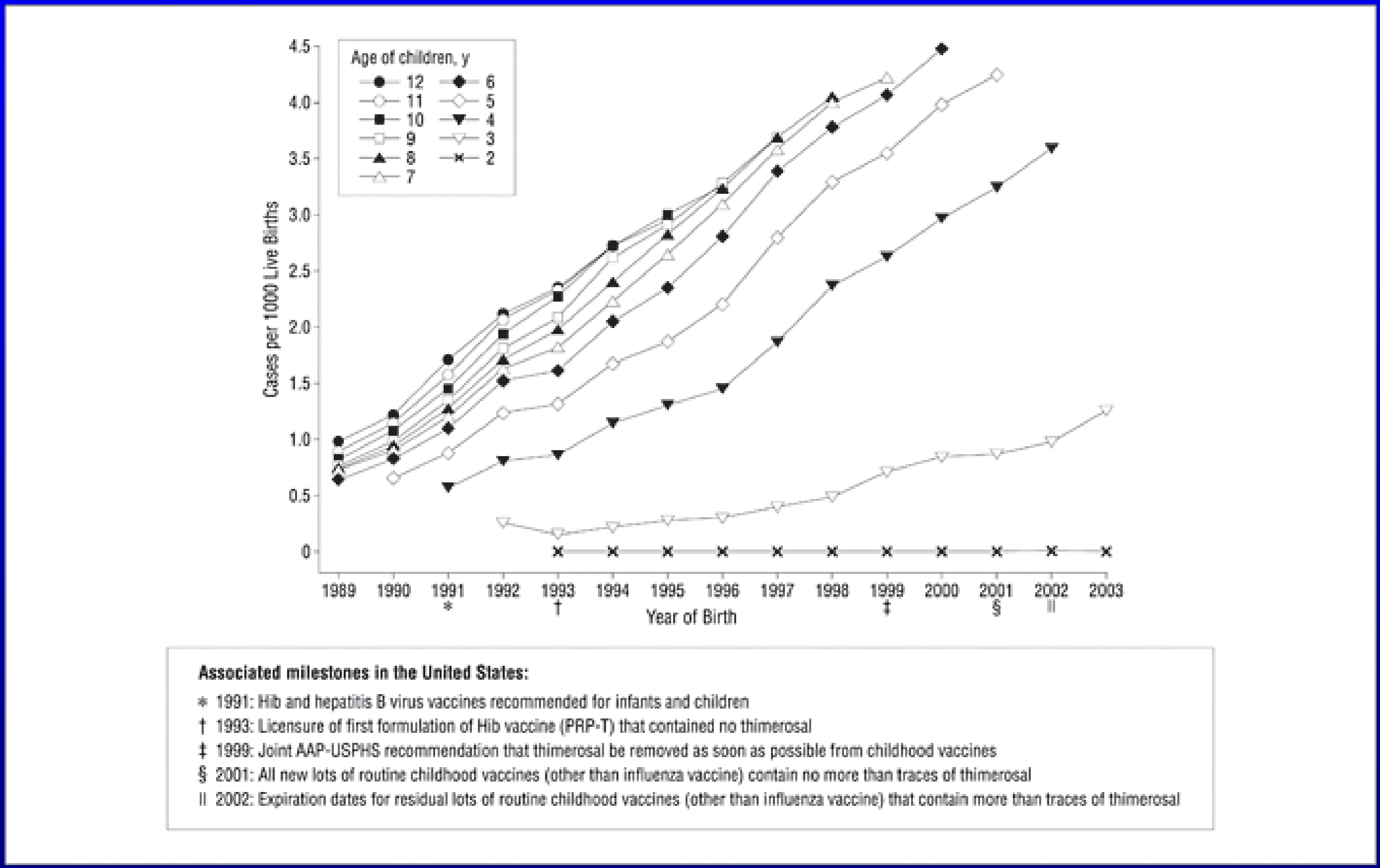
• The majority of autism cases are a result of *de novo* mutations, occurring first in the parental germ line.

• For reasons yet to be determined, female offspring are considerably more resistant to displaying the effects of such mutations than are males.

• Resistant individuals, but females in particular, carrying a mutation may marry and, with a probability of 50%, pass the mutation to their offspring, who will display the symptoms with high probability if male.

Is autism prevalence on the rise?

California's Developmental Services System
Schechter and Grether, 2008



Is this an increase in diagnosis, prevalence, or both?

Rett Syndrome

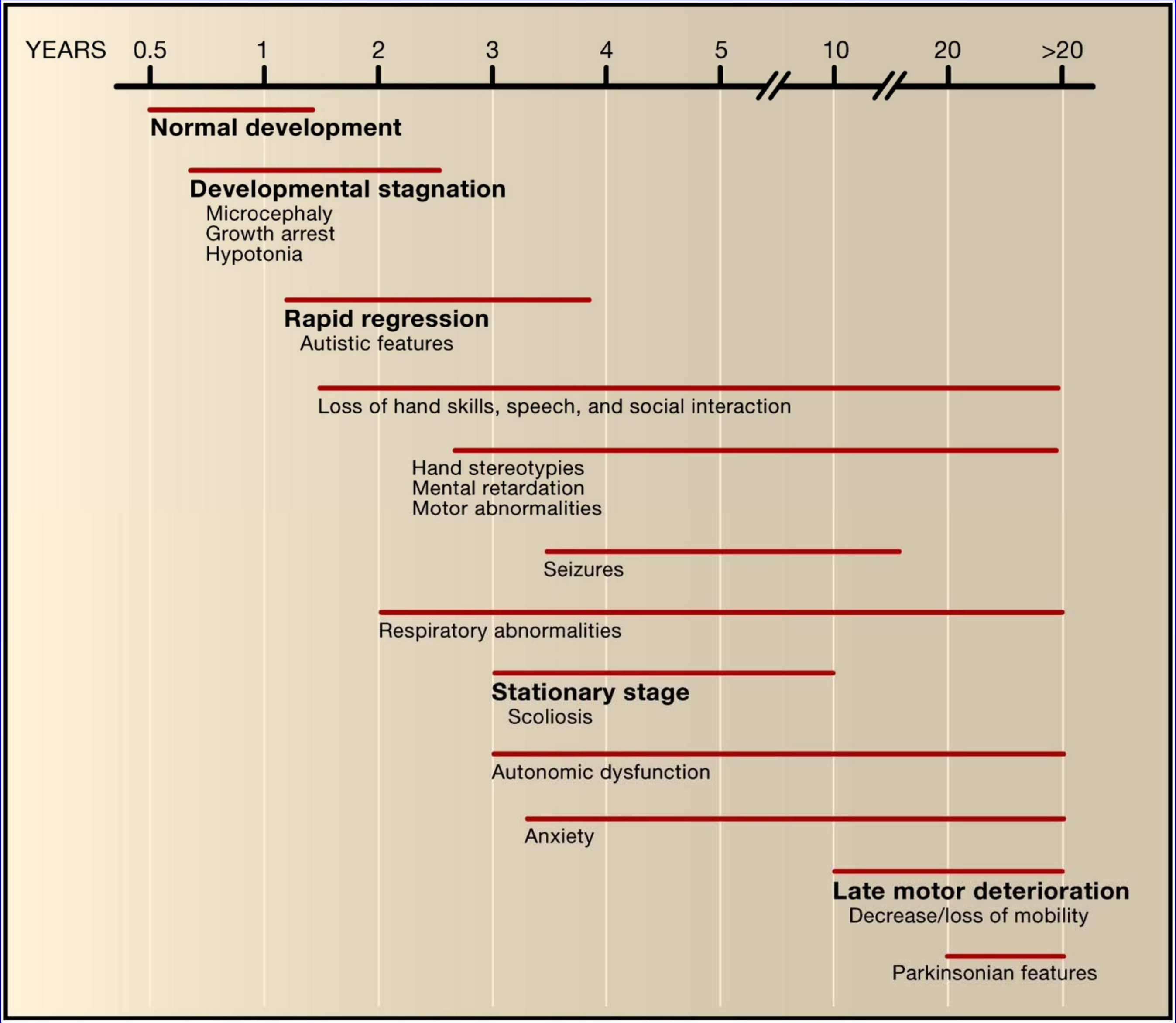
- Rett syndrome is the only one of the pervasive developmental disorders with a single known genetic cause
  - DSM IV Pervasive Developmental Disorders:
    - Autism
    - Asperger syndrome
    - Childhood disintegrative disorder
    - Rett syndrome
    - PDD-NOS

Rett Syndrome

- X-linked dominant, ~80% *MECP2* mutation
- ~1/10,000 in US population
- Neurodevelopmental regression around 6 to 18 months of age
- *MECP2* encodes a known epigenetic factor, methyl CpG binding protein 2

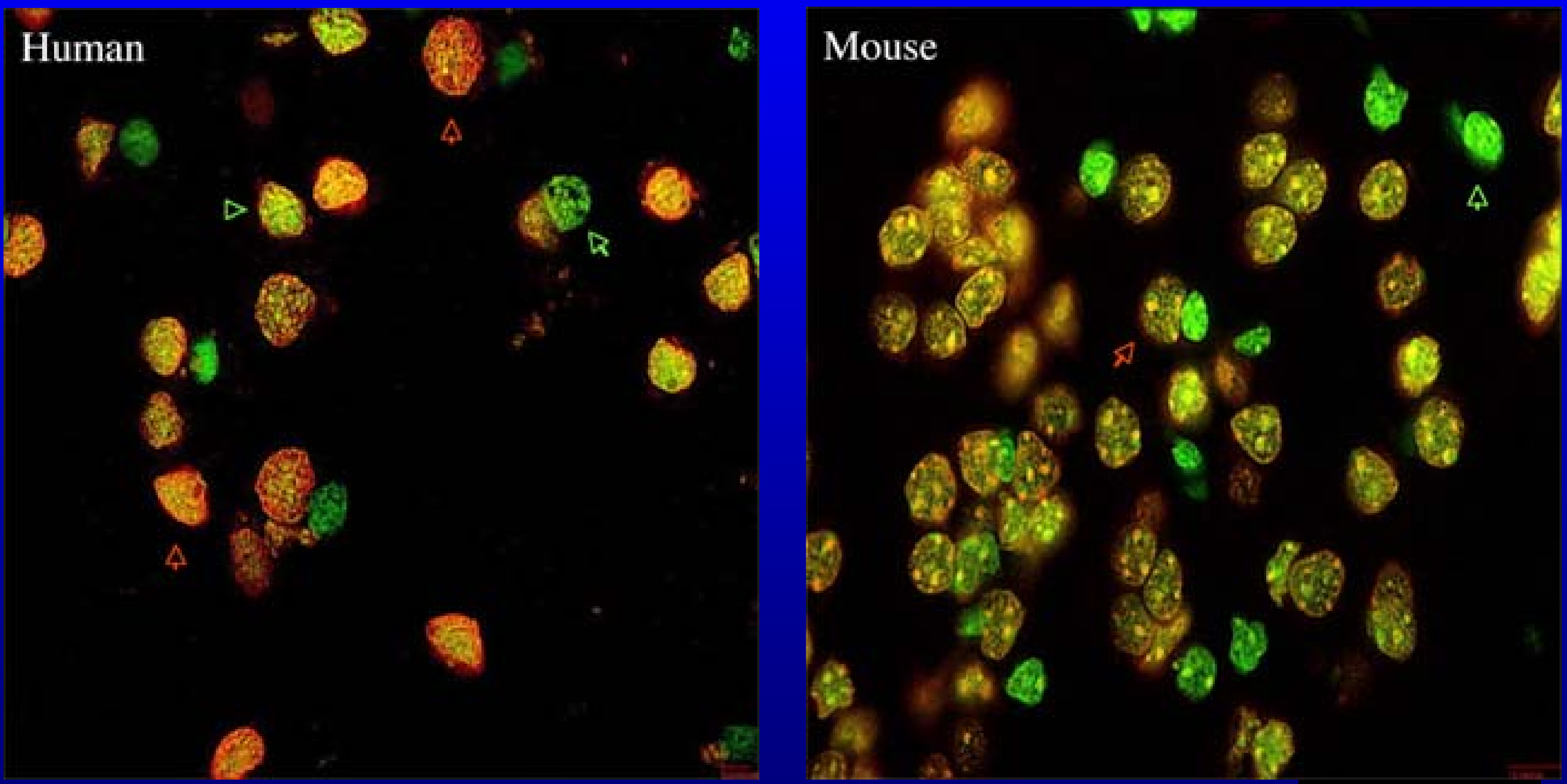
Rett syndrome involves epigenetics at 2 levels

Clinical Progression of Rett syndrome



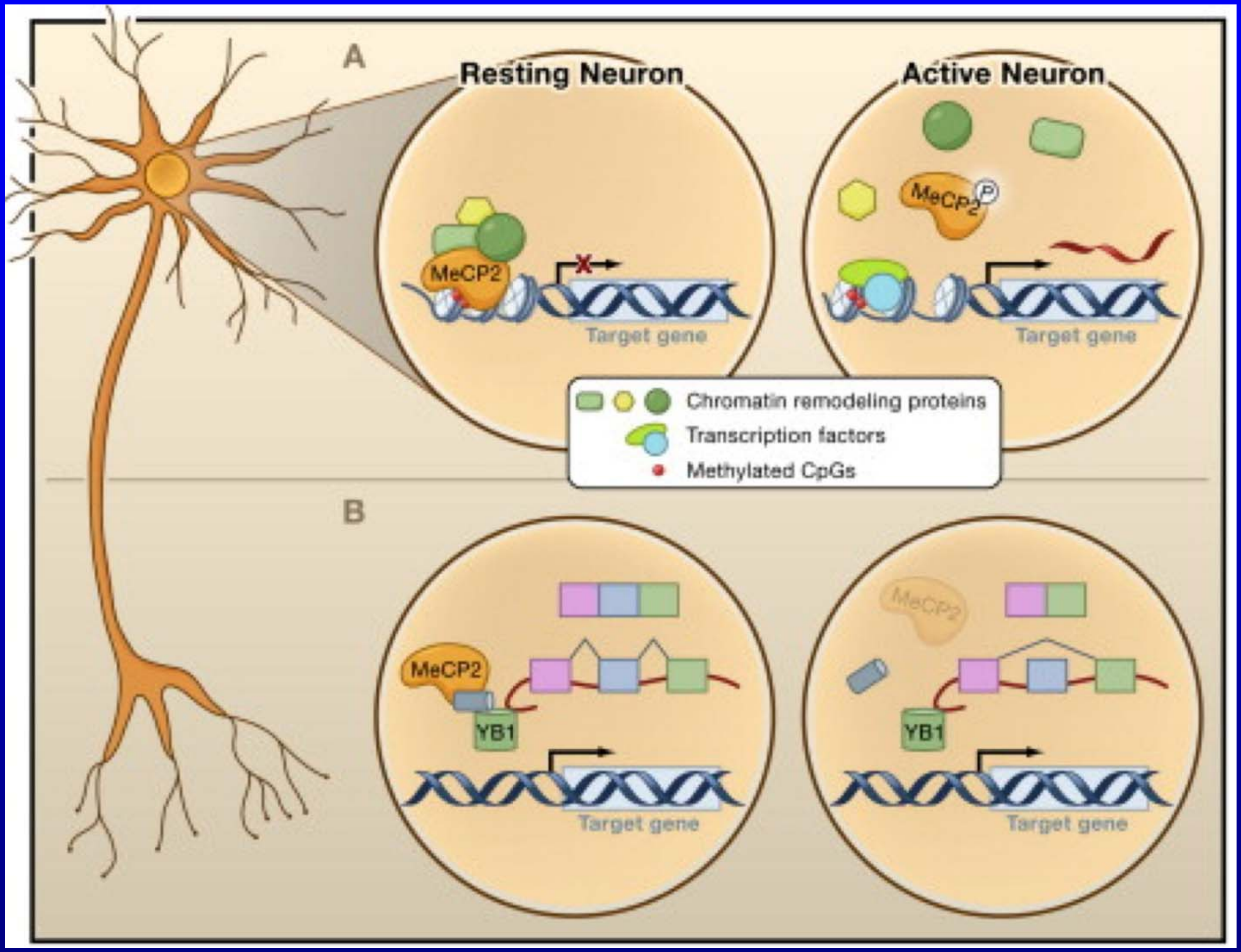
Chahrour and Zoghbi, Neuron, 2007

MeCP2 is a marker for mature neurons in the post-natal mammalian brain



Sytox Green
Anti-MeCP2

MeCP2 appears to have multiple roles in regulating gene expression in neurons



Activity dependent gene regulation

Regulation of alternative splicing

Chahrour and Zoghbi, Neuron, 2007

Genetic and environmental interactions in regressive autism

What Rett syndrome reveals

Rett syndrome
MECP2 mt

In utero	Normal infancy	Regression	Autism/MR

Environmental exposures

Regressive autism
de novo CNV?
Genetic susceptibility

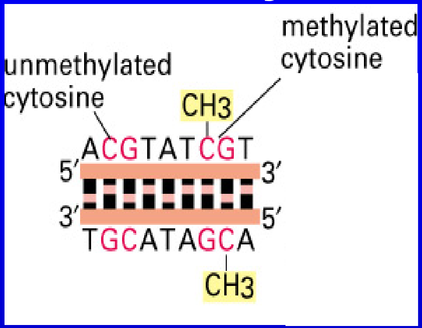
In utero	Normal infancy	Regression	Autism/MR

Etiologic environmental exposures in autism could be causitive (thalidamide, valproate, Rubella), or additive to genetic susceptibility (likely to be more common)

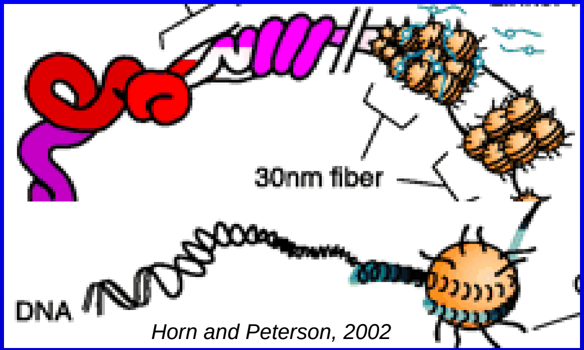
Long-term effects from in utero exposures could alter epigenetic mechanisms, leading to behavior and cognitive dysfunction in the child and adult

Epigenetics

DNA methylation



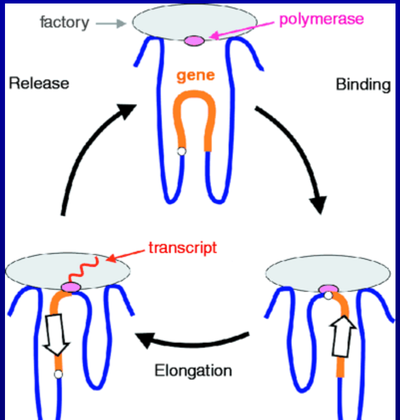
Histone modifications



Horn and Peterson, 2002

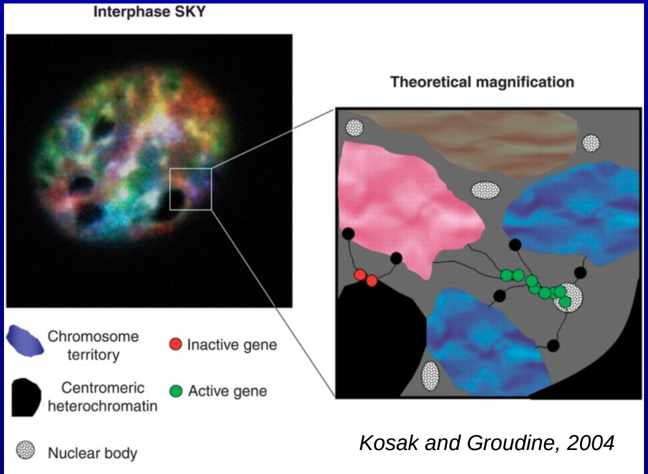
Inherited and reversible modifications to nucleotides or chromosomes that do not change the sequence but can alter gene expression

Chromatin structure



Cook, 1999

Spatial organization of chromosomes



Kosak and Groudine, 2004

Epigenetics

Examples of epigenetic mechanisms

X chromosome inactivation

Calico cats are females and are mosaics of cells expressing black and orange coat colors

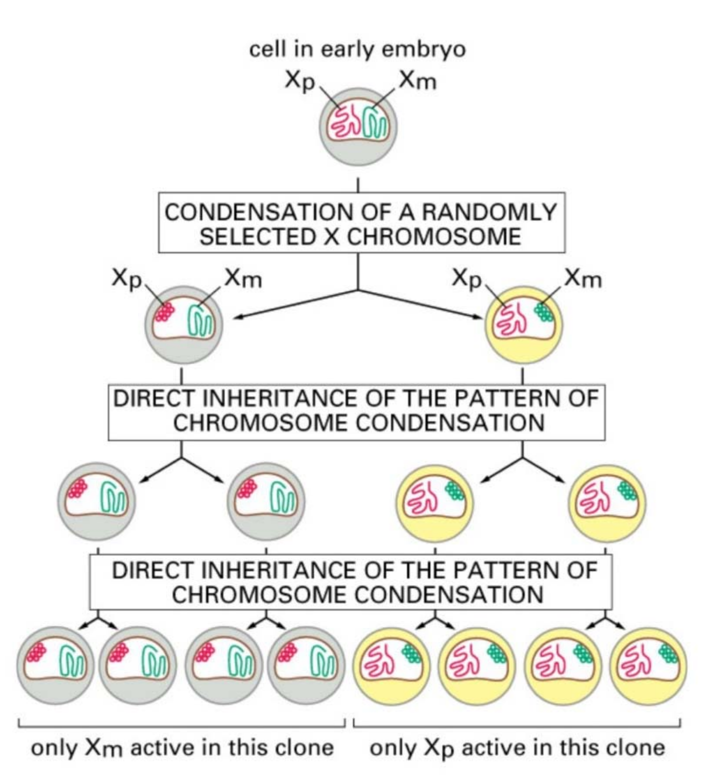
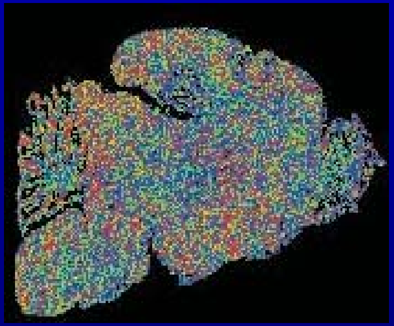


Figure 7–77. Molecular Biology of the Cell, 4th Edition.

Rett syndrome



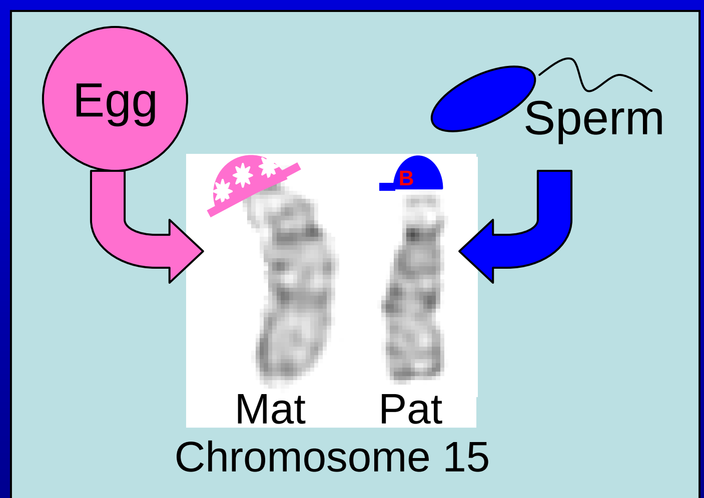
Rett girls are mosaics of cells expressing mutant *MECP2*

Epigenetics

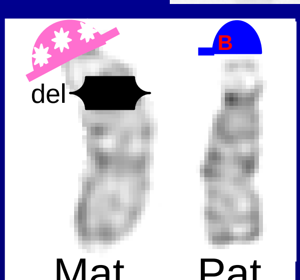
Examples of epigenetic mechanisms

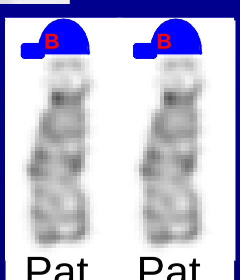
Parental Imprinting

Angelman syndrome

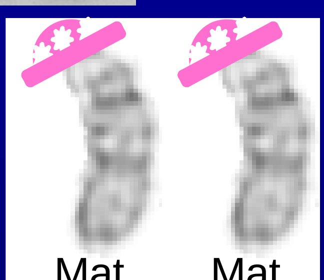


Prader-Willi syndrome





15q11-13



Epigenetics

Examples of epigenetic mechanisms

Tissue-specific and developmental differences in gene expression

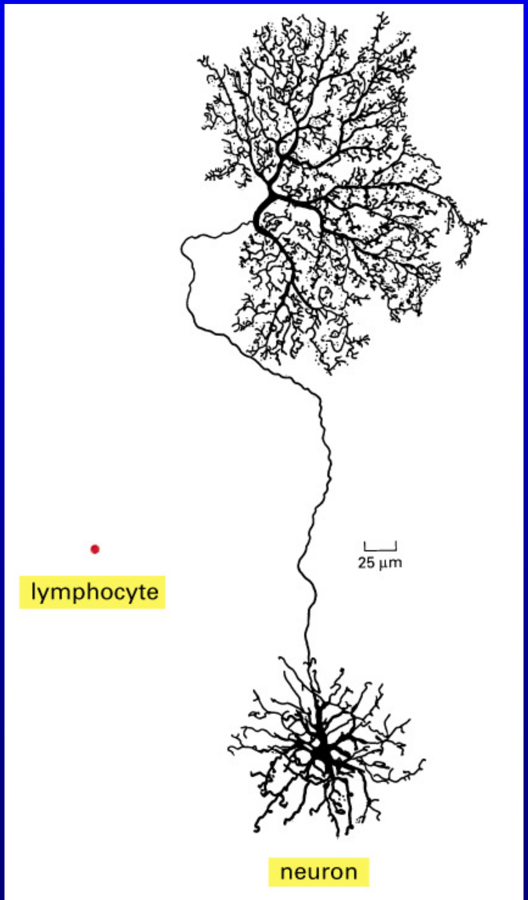
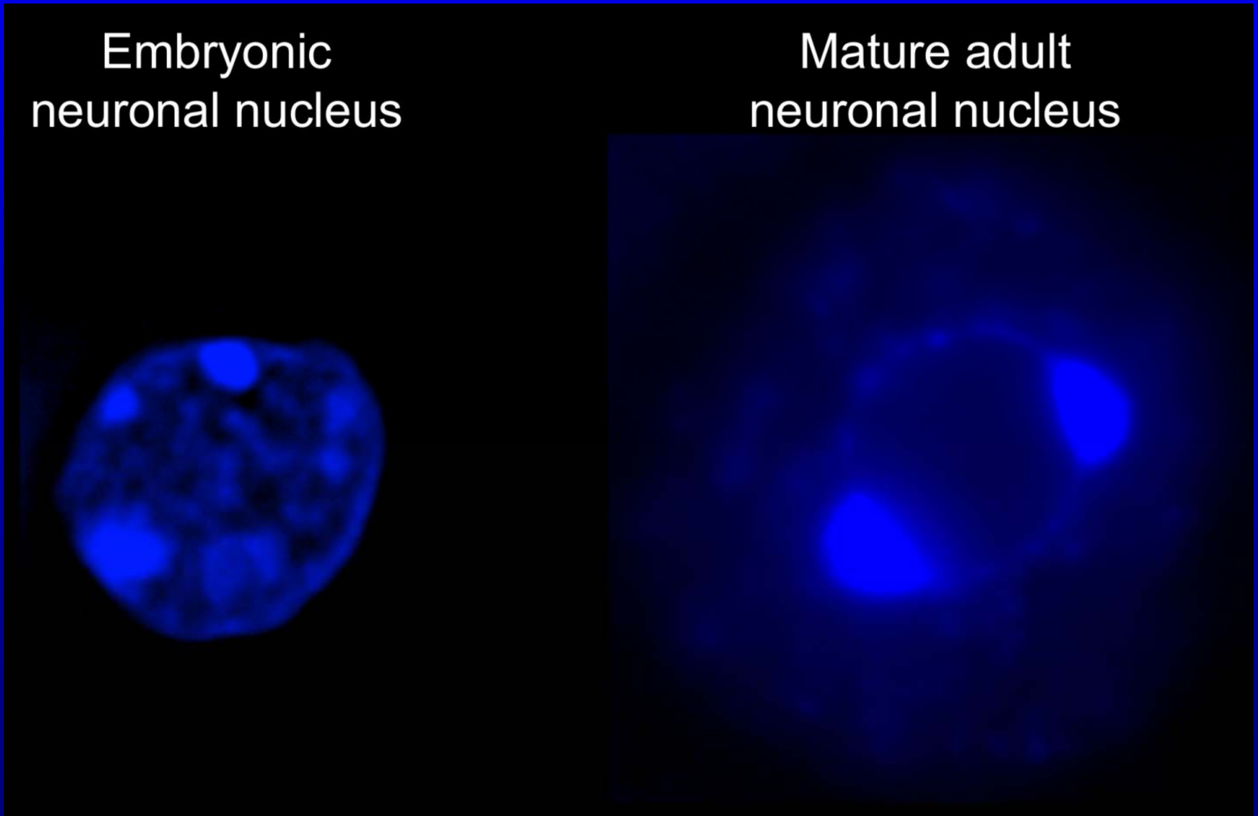


Figure 7–1. Molecular Biology of the Cell, 4th Edition.

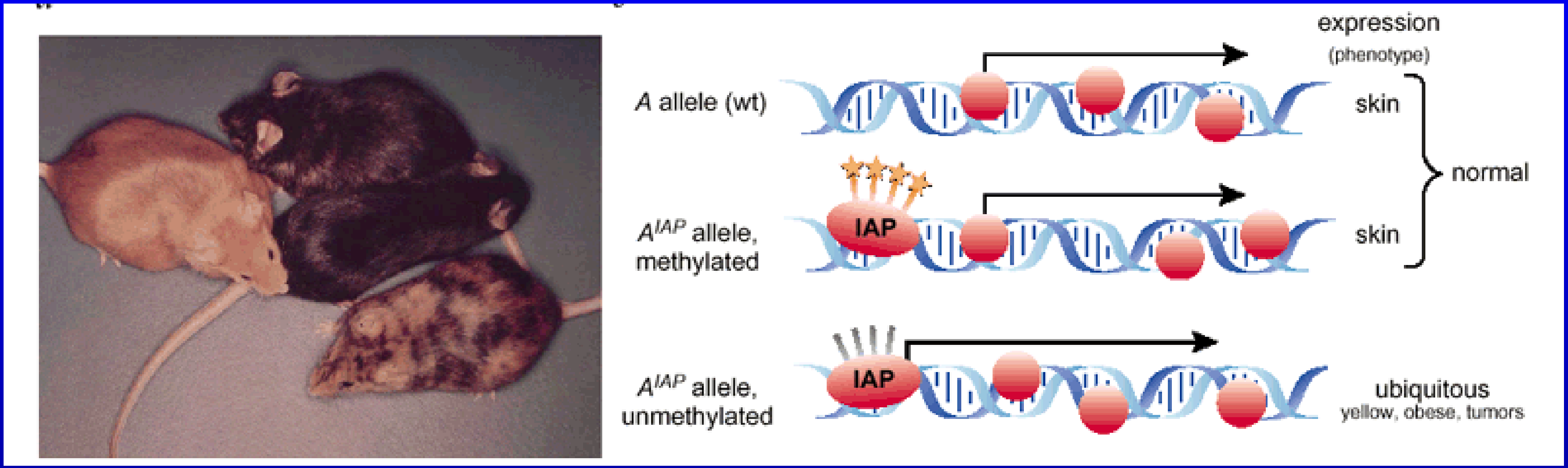


DNA dye (DAPI) of mouse cortical neurons

Epigenetics

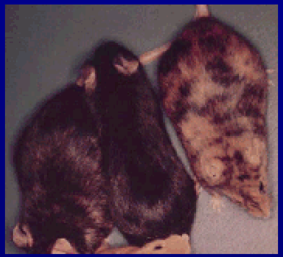
<u>Examples of epigenetic mechanisms</u>

Environmental effects on gene expression



Bisphenol A (BPA)

Bisphenol A (BPA) + folic acid



Dolinoy et al, PNAS, 2007

Epigenetic disorders on the autism spectrum

- The imprinted disorders Prader-willi and Angelman syndromes are on the autism spectrum.
  - 2-42% of AS and PWS cases have comorbid autism, depending on study
  - Uniparental disomy cases of PWS may be more frequently autistic

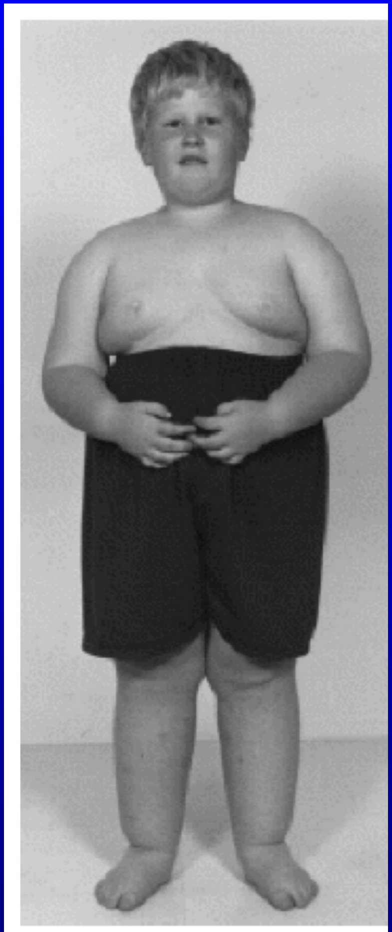
- Maternal 15q11-13 duplications are the most common cytogenetic cause of autism (1-3%)

Angelman and Prader-Willi syndromes

Imprinted disorders caused by 15q11-13 deletions or deficiency (~1/20,000)

AS: Maternal 15q11-13 deletion, paternal disomy, maternal UBE3A mutation, imprinting defects

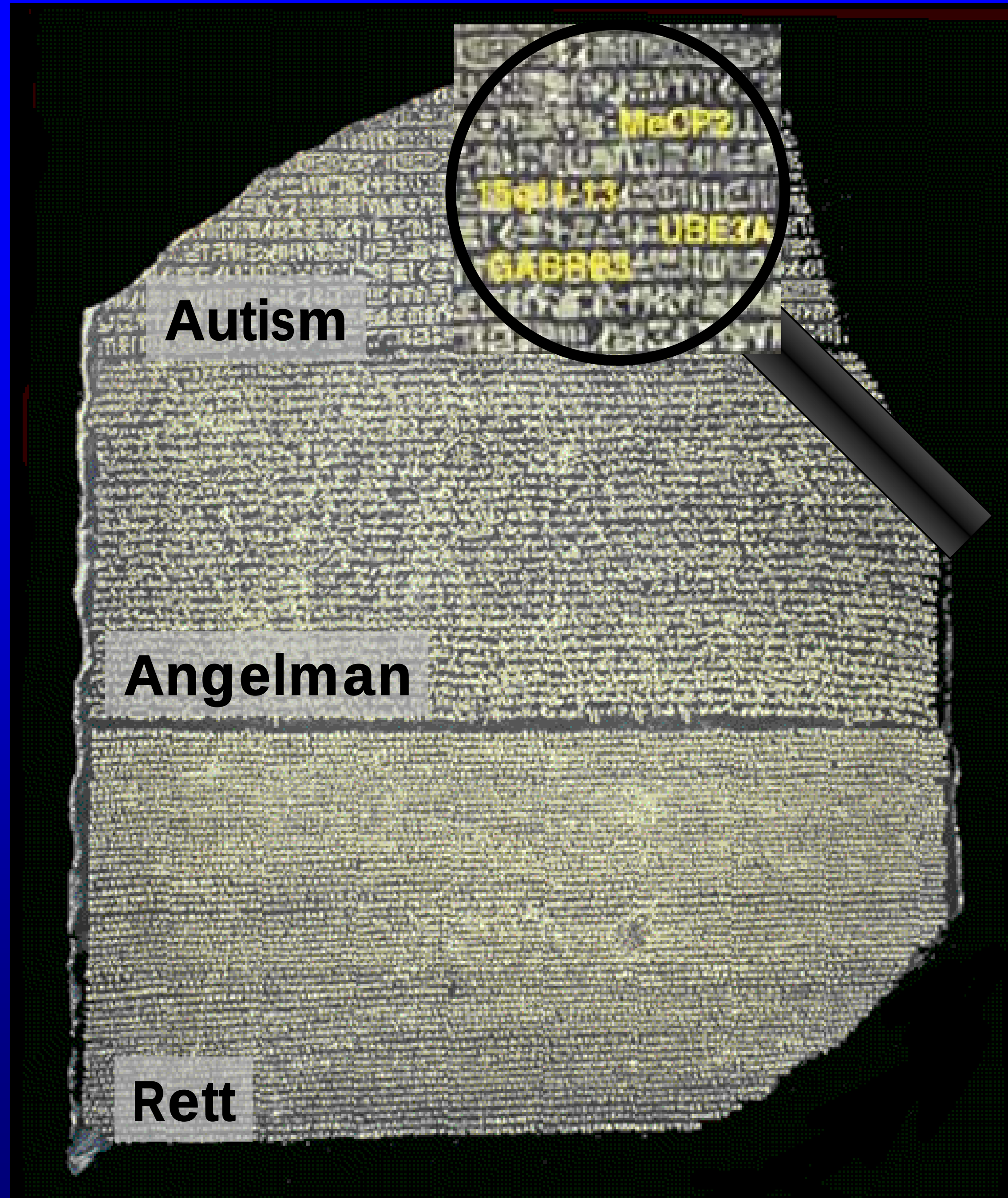
PWS: Paternal 15q11-13 deletion, maternal disomy, imprinting defects



Parental Imprinting and Mammalian Reproductive Technologies

- Many cloned livestock exhibit “large offspring syndrome” due to dysregulated expression of Igf2.
- Cloned mice and embryonic stem cells have many epigenetic defects in imprinted genes.
- Human ES cell lines exhibit altered methylation patterns compared to normal human tissue.
- Human children from in vitro fertilization (IVF) have increased rates of Angelman and Beckwith-Wiedemann syndromes.

The Rosetta Stone approach to "decoding" the complex genetics and epigenetics in autism



Evidence for epigenetic overlap between autism, RTT, and AS

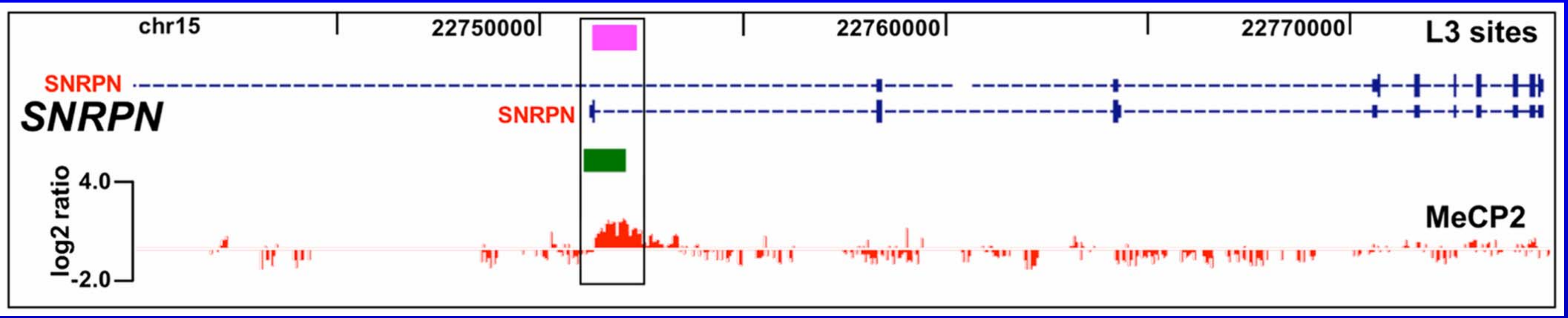
- MeCP2 expression is significantly reduced in 79% of autism post-mortem brain samples
- Methylation of the *MECP2* promoter correlates with reduced expression in male autism brain samples
- GABRB3 expression (15q11-13) is significantly reduced in 56% of autism post-mortem brain samples
- Biallelic expression levels of GABRB3 are epigenetically dysregulated in Rett and autism postmortem brain
- Homologous pairing of 15q11-13 in mature neurons is deficient in RTT, autism, and AS

Ravi Nagarajan
GGG student

Amber Hogart
GGG student

Karen Thatcher
GGG student

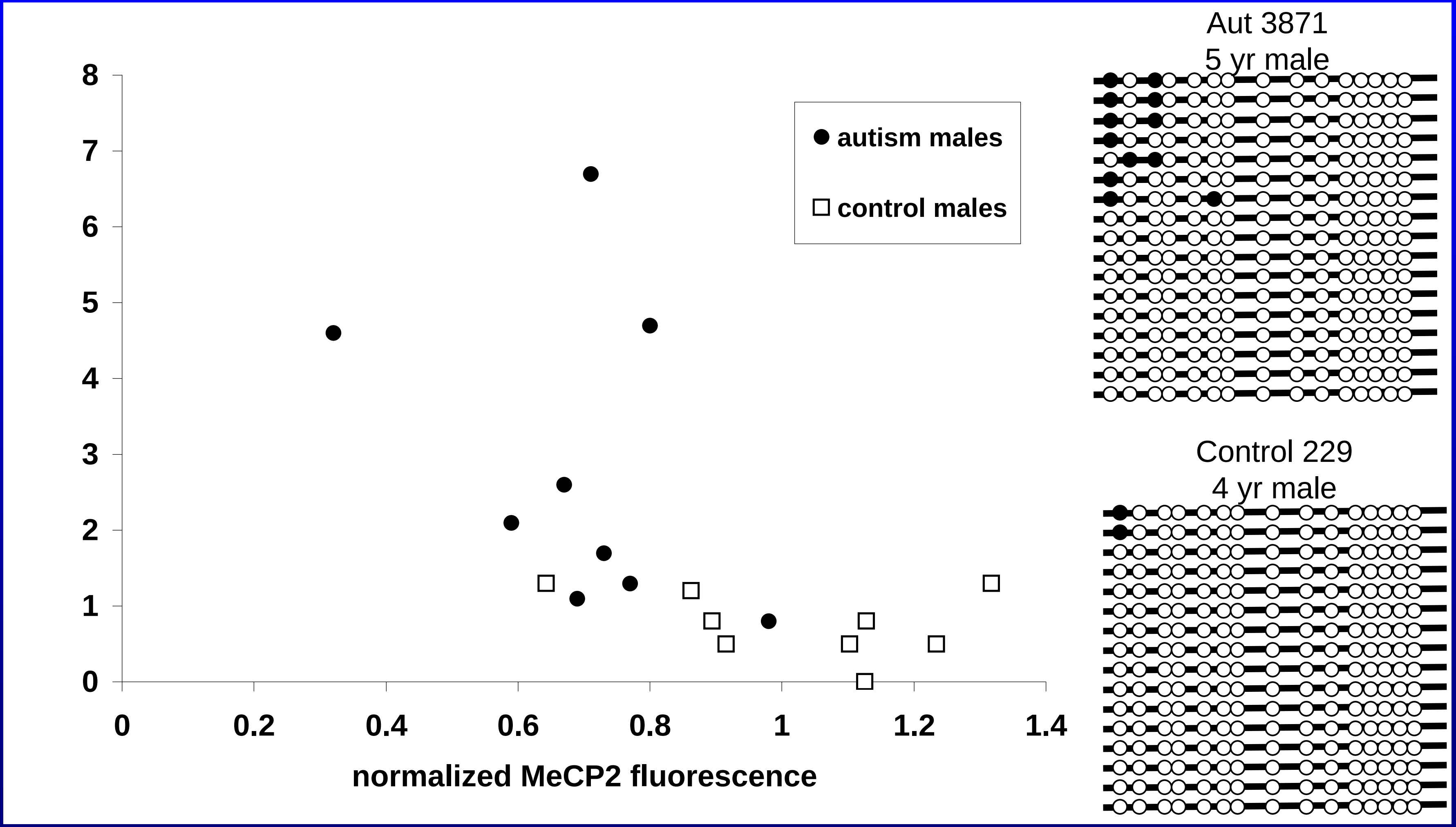
MeCP2 binds to the imprinting control region of 15q11-13 and regulates *UBE3A* and *GABRB3* expression



ChIP-chip analysis of MeCP2 binding at *SNRPN* and 62 additional sites within 13 MB of 15q11-13 *Yasui et al., 2007*

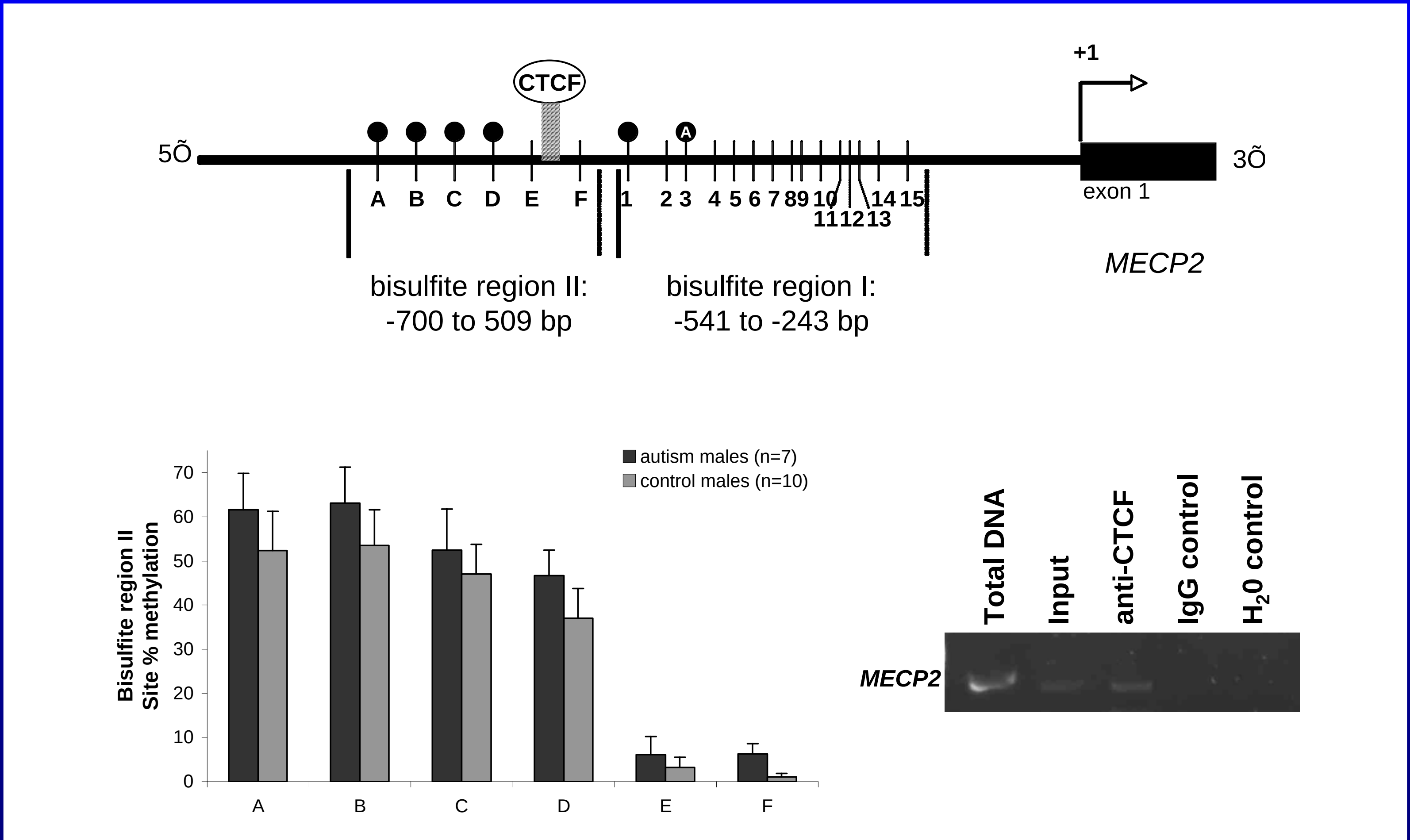
MECP2 mutation or deficiency does not alter imprinted expression, but reduces levels of *UBE3A* and *GABRB3*
Samaco et al, 2005

Reduced MeCP2 in autism frontal cortex correlates with aberrant methylation



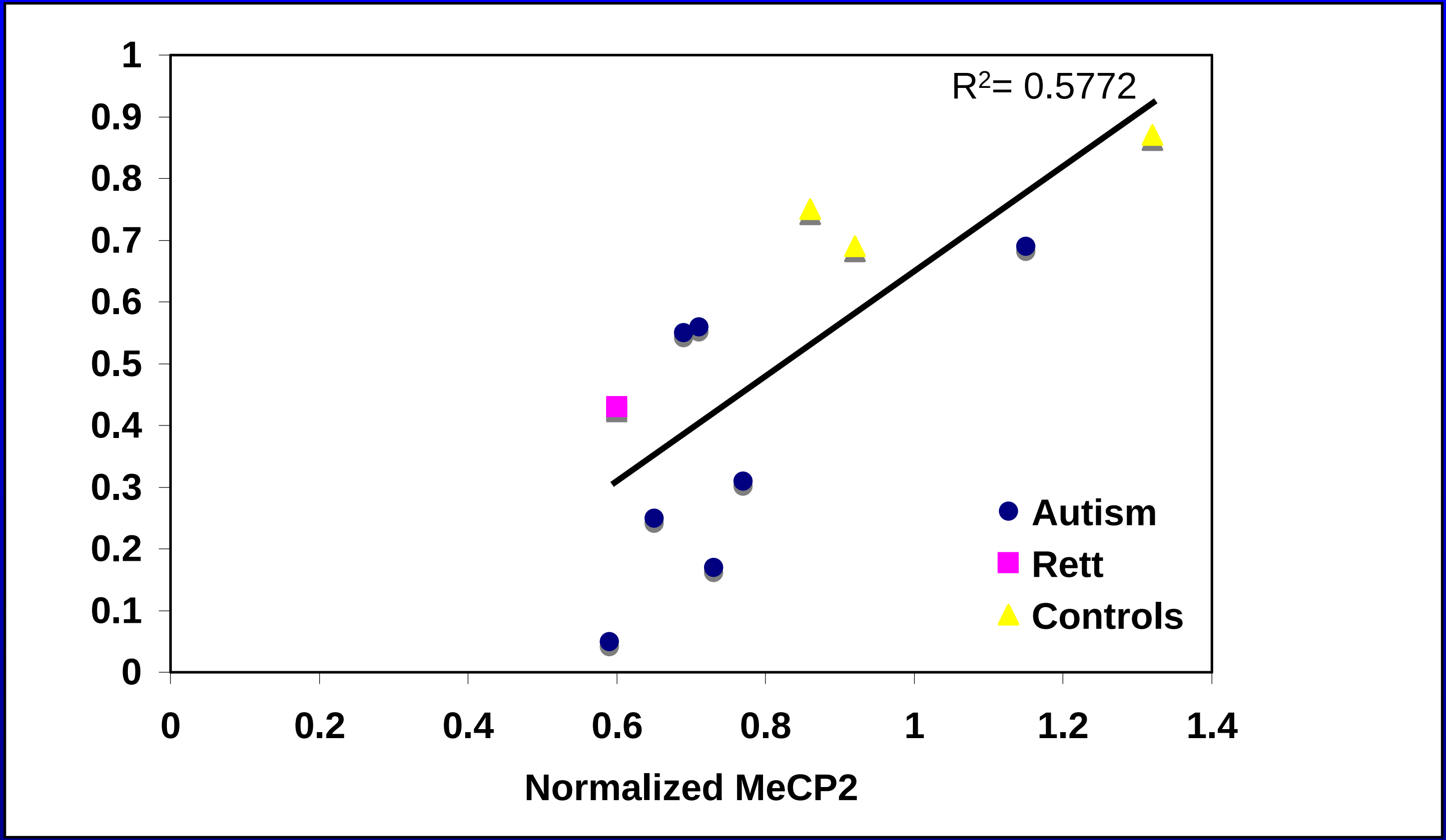
Nagarajan et al, Epigenetics, 2006

Identification of a methylation boundary element upstream of *MECP2* bound by CTCF



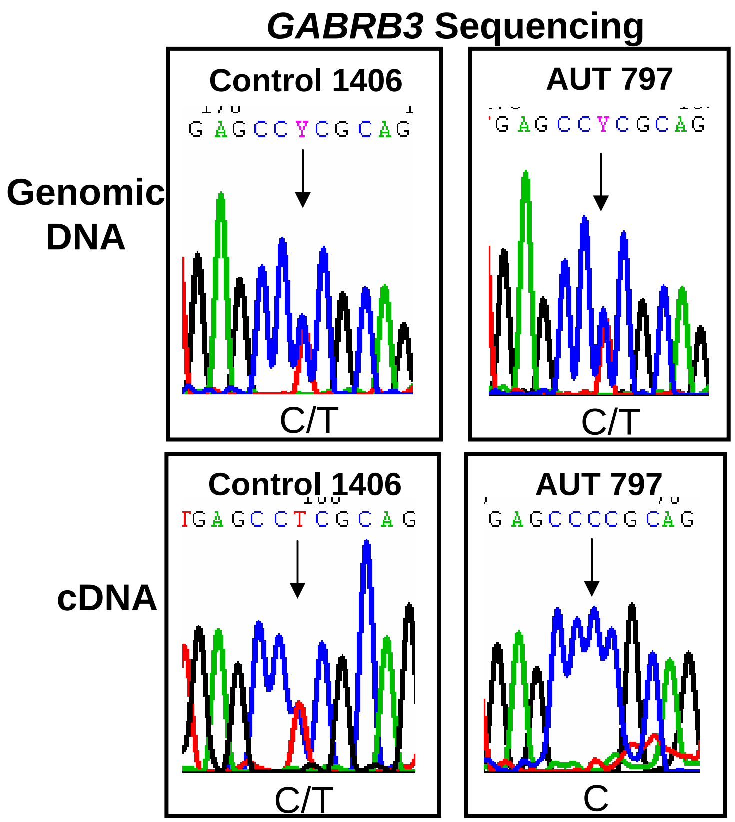
Nagarajan et al, Autism Research, in revision

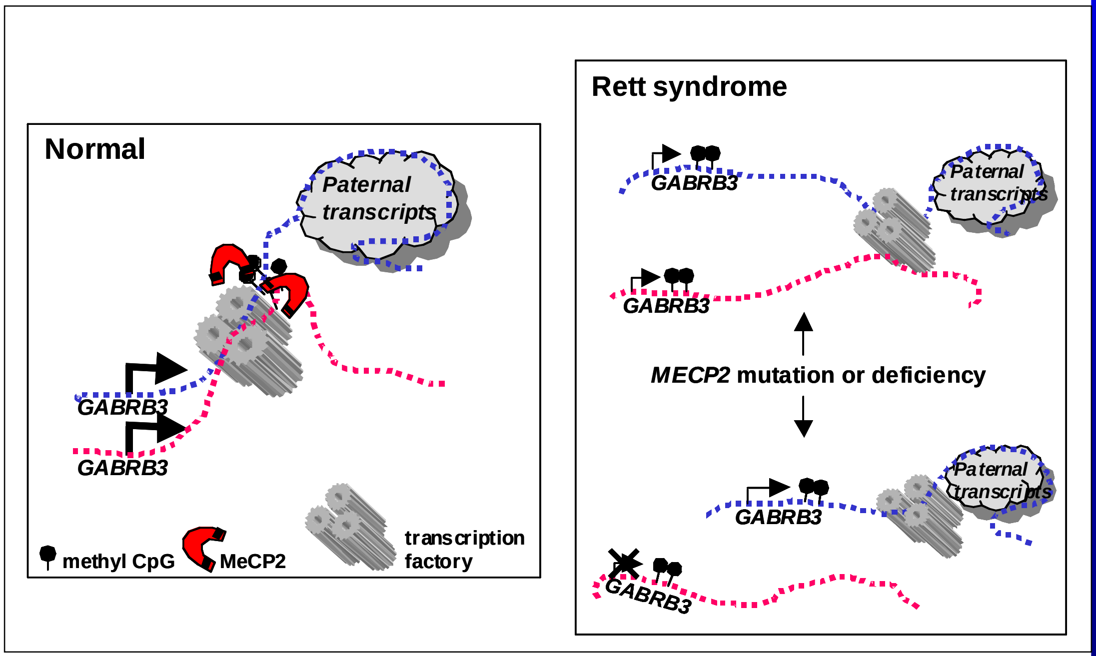
GABRB3 expression positively correlates with MeCP2



Significant correlation between MeCP2 and GABRB3 protein levels suggests that MeCP2 positively regulates *GABRB3* expression

Nonimprinted *GABRB3* is epigenetically dysregulated in a subset of autism and Rett syndrome brains

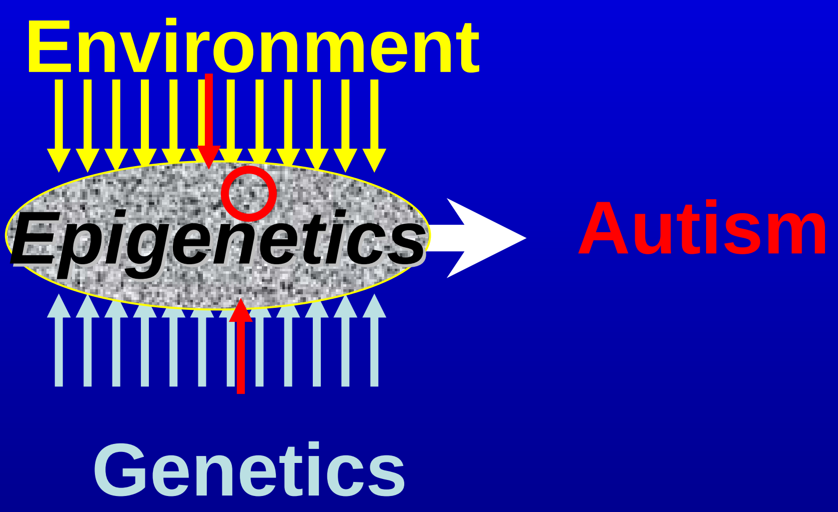




Hogart et al, Hum. Mol. Genet., 2007

What is the future for epigenetics and autism?

Defining precise genetic and environmental risk factors and develop tests for precise epigenetic alterations



Future directions examining environmental pollutants on epigenetics in neurodevelopment

BDE-47 **PCB-95**

GABRB3 ***MeCP2*** ***UBE3A***

Social behavior
Cognition
Seizures

Mecp2$^{\mathbf{308/+}}$
Mecp2$^{\mathbf{308/y}}$

Animal model component

Human subject component

Epigenetic interaction of MECP2 and organic pollutants in neurodevelopment

Perinatal exposure
BDE-47

4 w prior/ 3 w in utero/ 3 w lactation

0.03 mg/kg/day
1 mg/kg/day
vehicle control

x

Mecp2$^{308/+}$ C57Bl6/J

12 different
treatment x genotype
categories

Mecp2$^{+/+}$

Mecp2$^{308/+}$

Mecp2$^{+/y}$

Mecp2$^{308/y}$

Test perinatally exposed mice for social and cognitive behavior
Test mouse brains for epigenetic changes in MeCP2, UBE3A, global DNA methylation and histone modifications, etc

Behavioral Testing

Growth & Reflex Assessment

Ultrasonic Vocalization Measurement

Sociability Test

Social Dyadic Interaction

Acoustic Startle and Pre-Pulse Inhibition test

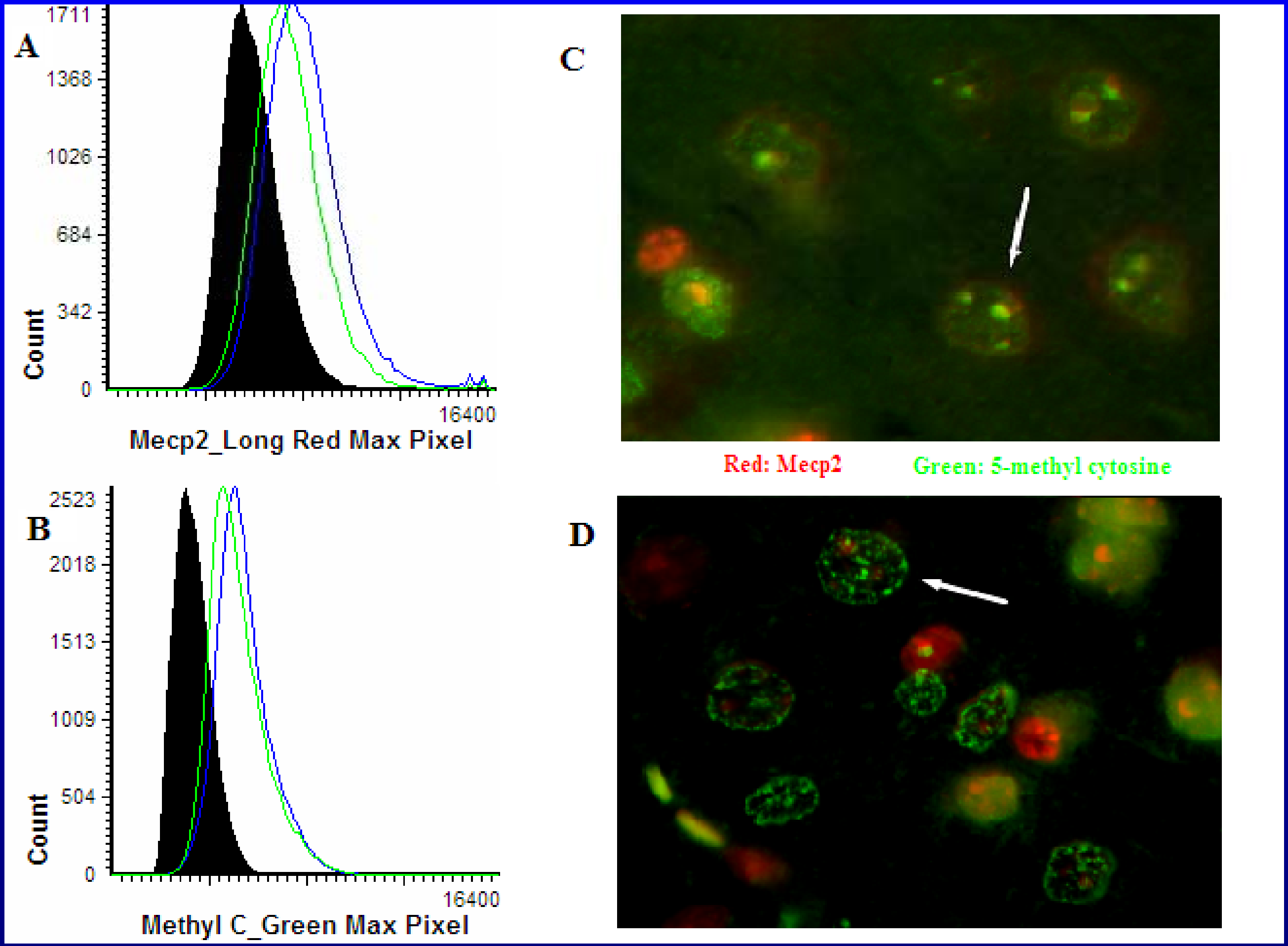
Social Transmission of Food Preference

Elevated Plus Maze

Locomotor Activity Integra

Spatial Memory and Learning in the Water Maze

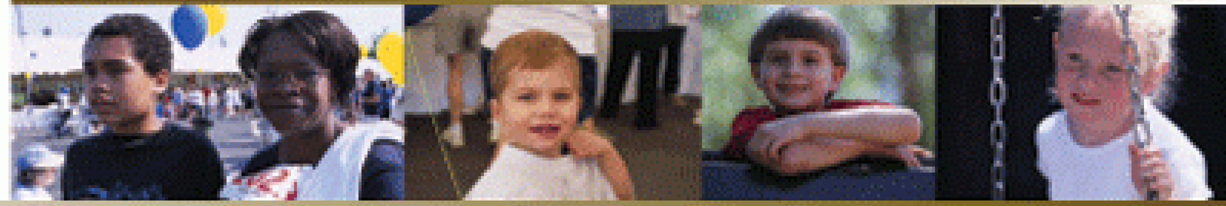
Preliminary evidence of epigenetic changes with perinatal BDE-47 exposure



CHARGE

CHILDHOOD AUTISM RISKS FROM GENETICS AND THE ENVIRONMENT

BeInCHARGE@ucdavis.edu



Irva Hertz-Picciotto, PI

Comprehensive, collaborative evaluation of autism

- Medical evaluations
- Environmental exposures/epidemiology
- Behavior and neuropsychology
- Genomics
- Brain structure/imaging
- Immune function
- Epigenetics

DNA samples from four diagnostic categories

- Early onset autism
- Regressive autism
- Developmental delay
- Typically developing controls

Parental DNA also available

Epigenetic analyses on human samples

CHARGE blood DNA samples

- X chromosome inactivation
- DNA methylation at chromosome 15 imprinting control regions
- *MECP2* promoter methylation

Human postmortem brain samples

- X chromosome inactivation
- DNA methylation at chromosome 15 imprinting control regions
- *MECP2* promoter methylation
- MeCP2 and GABRB3 expression

Correlate epigenetic changes with PBDE tissue levels

No evidence for X chromosome inactivation skewing differences between mothers of males with autism

	n=	number of uninformative samples (%)	avg age (y)[a]	avg small allele size (bp)[a]	avg large allele size (bp)[a]	avg % skewing[a,b]	avg inactive allele size (bp)[a,c]	% mothers with < 5% or > 95% skewing[a]	% mothers with < 15% or > 85% skewing[a]
Typical Development	23	5 (22)	33	274	286	53	280	11	17
Delayed Development	24	3 (13)	32	275	287	45	282	0	10
Autism	27	2 (7)	35	276	288	46	284	8	16
ASD	25	3 (12)	36	277	286	60	280	9	18

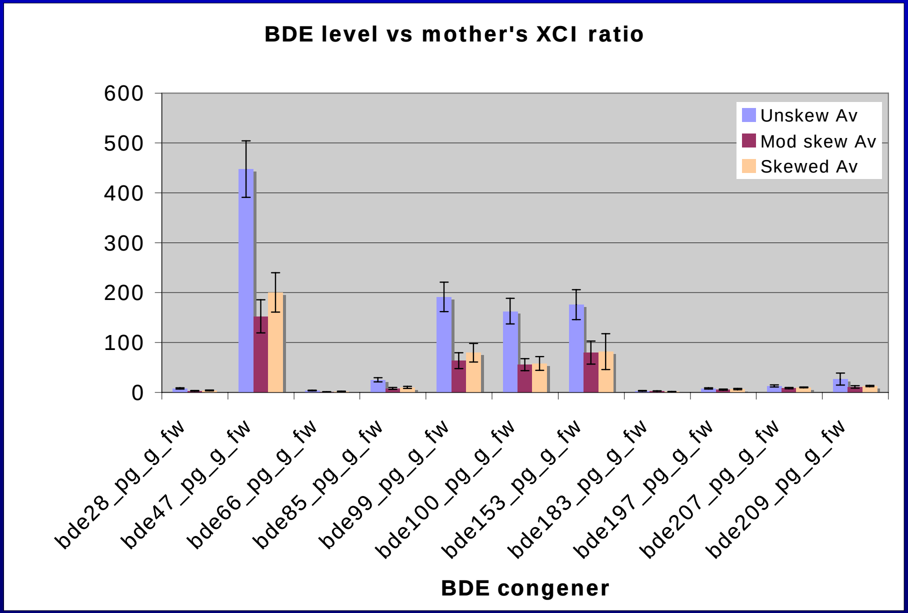
Notes:

[a] - for informative samples

[b] - percent of cells with small allele inactive

[c] - average of allele sizes (bp) of the alleles that are inactivated > 50%

Nagarajan et al, Autism Research, in revision



PBDEs protective for XCI skewing?

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Human tissue samples
CHARGE
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